Kidney International, Vol. 56 (1999), pp. 1274–1276

Effect of oxidant stress on growth factor stimulation of proliferation in cultured human proximal tubule cells

Charles E. Weston, Morgan B. Feibelman, Kuoting Wu, and Eric E. Simon

Department of Medicine, Tulane University School of Medicine and the Veterans Administration Hospital, New Orleans, Louisiana, USA

Effect of oxidant stress on growth factor stimulation of proliferation in cultured human proximal tubule cells. Restoring kidney function after injury involves cell migration and proliferation, processes that are yet to be precisely defined. Because epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) promote recovery from acute renal failure, we used SV-40 immortalized human proximal tubule cells to examine the effects of these growth factors on cell proliferation after peroxide injury (1.5 mM for 1 hour). ATP levels decreased to ~15% of control values immediately after injury but returned to nearly normal levels after 4 hours of recovery. Under control conditions, both EGF and IGF-1 stimulated proliferation and their effects were additive. However, 20–24 hours after injury, while IGF-1 stimulated proliferation, EGF was no longer effective nor was the combination of EGF and IGF-1. Although the EGF receptor was decreased 20 hours after injury, the lack of IGF-1 effect could not be explained by loss of the IGF-1 receptor, which remained unchanged after peroxide injury. Thus, the mechanism responsible for the blunting of the IGF-1 effect on proliferation following injury remains speculative. However, we conclude that the effects of growth factors under control conditions may not predict their effects after injury.

The events involved in the recovery of tubule epithelial cells from acute injury remain incompletely defined. During the recovery phase, cells lining the tubule lumen first spread to cover the defects caused by the loss of tubule cells during injury [1]. Cells undergo proliferation to replace those that were lost, and finally, the cells redifferentiate [2]. Our laboratory has been interested in the potential role of integrins in the recovery of proximal tubule cells from acute renal injury [3] because of their importance in cell spreading and migration [4, 5]. Furthermore, integrins influence the response to growth factors [6].

Our initial studies in JTC-12 cells, a monkey proximal tubule cell line, showed that after oxidative stress, the actin cytoskeleton was disrupted and cell adhesion was decreased [7]. By 24 hours after recovery, the actin cytoskeleton had largely reverted to normal, as had cell adhesion. During the period of recovery, integrin expression was only modestly and selectively increased, with an approximate 25 to 50% increase in α_6 but not β_1 or $\alpha_V\beta_3$ integrin subunits [7]. Thus, we reasoned that integrin signaling is likely to be more important than integrin expression *per se* in the recovery of cell function. To examine the influence of integrin signaling on the recovery of cells from acute injury and the interaction of one of the key signaling molecules, focal adhesion kinase [8], with growth factors, we first needed to define the role of growth factors in our system in one of their most basic functions: cell proliferation.

Both epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) accelerate recovery from acute renal failure in experimental animals [9]. EGF has been more extensively studied and promotes recovery from various forms renal failure, including ischemic [10] and toxic acute renal failure, such as following mercuric chloride [11] and gentamicin [12] exposure. The mechanisms underlying the beneficial effects of the growth factors are not completely understood. The stimulation of cell proliferation is one potential mediator of these effects. Indeed, in the first study examining the beneficial effects of growth factors on recovery from renal insult, Humes et al showed that EGF increased proliferation in the rat renal cortex following ischemia [10]. Furthermore, it is known that both EGF and IGF-1 stimulate proliferation of various renal tubule epithelial cells in culture, including proximal tubule cells [13–16]. However, growth factor stimulation of epithelial cell migration is likely also important as EGF stimulates the migration of intestinal epithelial cells in an extracellular matrix-specific manner [17].

In some epithelial cells, the mitogenic effects of EGF and IGF-1 are additive or synergistic [18–20]. It has been postulated that the reason for the additivity is that they work on different points in the cell cycle. EGF may act as a so-called competence factor stimulating quiescent cells in the G0 phase to enter the cell cycle, whereas

Key words: cell proliferation, JTC-12 cells, acute renal failure, renal insult, oxidative stress.

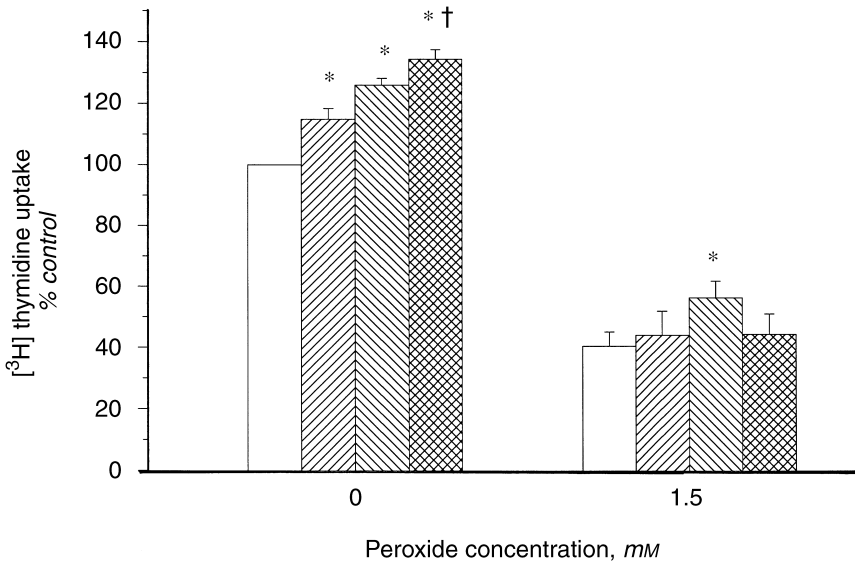

Fig. 1. Effects of epidermal growth factor (EGF) and insulin-like growth factor-1 (IGF-1) on proliferation between 20 and 24 hours after exposure to hydrogen peroxide. Symbols are: (□) no growth factors; (▨) EGF; (▧) IGF-1; (▩) EGF + IGF-1; *$P < 0.05$ vs. no growth factor addition after exposure to the same peroxide concentration; †$P < 0.025$ vs. EGF or IGF-1 treatment alone.

IGF-1 acts primarily in the G_1 to S phase transition, acting as a so-called progression factor [18–20]. However, EGF in some cells also stimulates G1 cells to progress to the S phase.

Studies on the effects of EGF and IGF-1 in cultured tubule epithelial cells following injury are limited. Some studies have suggested that after injury, EGF stimulates recovery [21, 22]. None of the studies have examined whether the effects of EGF and IGF-1 after injury might be additive.

For these studies, we then used SV-40 immortalized human proximal tubule cells when they became available from Dr. Lorraine Racusen [23]. The cells were injured with 1.5 mM peroxide for one hour. Following peroxide injury, adenosine 5'-triphosphate (ATP) levels fell to approximately 15% of normal, but within four hours, there was almost complete recovery of ATP levels.

Under control conditions (0 peroxide), as expected, both EGF and IGF-1 increased cell proliferation (Fig. 1). Furthermore, the combination of EGF and IGF-1 stimulated proliferation more than either growth factor alone. Between 20 and 24 hours after injury, proliferation was suppressed, but IGF-1 still stimulated proliferation. However, surprisingly, neither EGF nor the combination of EGF and IGF-1 stimulated proliferation over that seen in injured cells that had not been exposed to growth factors.

We examined the surface expression of EGF and IGF-1 receptors by flow cytometry. EGF receptor expression was significantly decreased by EGF (by about 60%) but not IGF-1. Furthermore, oxidant stress decreased EGF receptor expression (by approximately 35% after 20 hours recovery). Peroxide injury had no effect on IGF-1 receptor expression. In contrast to an *in vivo* study examining whole cortex [24], we found no increase in the EGF receptor after IGF-1, nor did EGF significantly alter the expression of the IGF-1 receptor.

The causes of the impaired proliferative response to EGF remain speculative, as does the apparent inhibition of the response to IGF-1. Certainly the decrease in EGF receptor expression after peroxide treatment could contribute to the decrease in EGF responsiveness. However, EGF had no effect on the IGF-1 receptor and thus cannot explain the blunting of the IGF-1 response by EGF. One possibility is that after oxidant stress, EGF alters IGF-1 receptor activation. Another possibility is that EGF alters IGF-1–binding proteins. The proximal tubule has been reported to produce IGFBP-2 [25], IGFBP-4 [25, 26], and IGFBP-5 [25] where they may be released or remain cell associated. By sequestering IGF-1, they play an important role in regulating the biological effects of IGF-1. Depending on the conditions, they may either inhibit or potentiate IGF-1 action. It is conceivable that EGF alters IGF-1 binding proteins after injury.

As noted earlier in this article, functionally, EGF and IGF-1 act on different points in the cell cycle. However, more recent studies suggest overlapping functions. For instance, depending on the cell type, both may increase cyclin D1 expression [27–30]. EGF, in some cases, may be growth inhibitory because of the stimulation of the cyclin kinase inhibitor $p21^{WAF/Cip1}$ [31–33]. The detailed effects of these growth factors in the proximal tubule are not known. It is conceivable that following injury, the effects of EGF and IGF-1 on cyclins, cyclin kinases, and/or cyclin kinase inhibitors differ from control conditions.

The extrapolation of the *in vitro* results to *in vivo* conditions is hazardous. However, we suggest that the

response to growth factors under control conditions may not predict their effects after injury.

ACKNOWLEDGMENTS

These studies were supported by a Grant-In-Aid from the American Heart Association, Louisiana Affiliate, a VA Merit Review grant, and a grant from the Dialysis Centers, Inc.

Reprint requests to Dr. Eric E. Simon, Section Nephrology SL45, Tulane University School Medicine, 1430 Tulane Avenue, New Orleans, Louisiana 70115, USA.
E-mail: esimon@tmcpop.tmc.tulane.edu

REFERENCES

1. Solez K: Acute renal failure (acute tubular necrosis, infarction, and cortical necrosis), in *Pathology of the Kidney*, edited by Heptinstall RH, Boston, Little, Brown, 1992, p 1235–1314
2. Bacallao R, Fine LG: Molecular events in the organization of renal tubular epithelium: From nephrogenesis to regeneration. *Am J Physiol* 257:F913–F924, 1989
3. Lefkowith JB, Simon EE: Adhesion receptors in renal development and disease, in *Molecular Biology of Cell Adhesion Molecules*, edited by Horton MA, Chichester, John Wiley & Sons, 1996, pp 155–174
4. Hynes RO: Integrins: Versatility, modulation, and signalling in cell adhesion. *Cell* 69:11–25, 1992
5. Ruoslahti E, Noble NA, Kagami S, Border WA: Integrins. *Kidney Int* 45(Suppl 44):S17–S22, 1994
6. Clark EA, Brugge JS: Integrins and signal transduction pathways: The road taken. *Nature* 268:233–239, 1995
7. Nigam S, Weston CE, Liu CH, Simon EE: The actin cytoskeleton and integrin expression in the recovery of cell adhesion following oxidant stress to a proximal tubule cell line (JTC-12). *J Am Soc Nephrol* 9:1787–1797, 1998
8. Ilic D, Damsky CH, Yamamoto T: Focal adhesion kinase: At the crossroads of signal transduction. *J Cell Sci* 110:401–407, 1997
9. Hammerman MR, Miller SB: Therapeutic use of growth factors in renal failure. *J Am Soc Nephrol* 5:1–11, 1994
10. Humes HD, Cieslinski DA, Coimbra TM, Messana JM, Galvao C: Epidermal growth factor enhances renal tubule cell regeneration and repair and accelerates the recovery of renal function in postischemic acute renal failure. *J Clin Invest* 84:1757–1761, 1989
11. Coimbra TM, Cieslinski DA, Humes HD: Epidermal growth factor accelerates renal repair in mercuric chloride nephrotoxicity. *Am J Physiol* 259:F438–F443, 1990
12. Morin NJ, Laurent G, Nonclercq D, Toubeau G, Heuson-Stiennon JA, Bergeron MG, Beauchamp D: Epidermal growth factor accelerates renal tissue repair in a model of gentamicin nephrotoxicity in rats. *Am J Physiol* 263:F806–F811, 1992
13. Humes HD, Beals TF, Cieslinski DA, Sanchez IO, Page TP: Effects of transforming growth factor-beta, transforming growth factor-alpha, and other growth factors on renal proximal tubule cells. *Lab Invest* 64:538–545, 1991
14. Franch HA, Shay JW, Alpern RJ, Preisig PA: Involvement of the pRB family in TGFβ-dependent epithelial cell hypertrophy. *J Cell Biol* 129:245–254, 1995
15. Zhang GH, Ichimura T, Wallin A, Kan M, Stevens JL: Regulation of rat proximal tubule epithelial cell growth by fibroblasts and growth factors, insulin-like growth factor-1 and transforming growth factor-beta, and analysis of fibroblast growth factors in rat kidney. *J Cell Physiol* 148:295–305, 1991
16. Blazer-Yost BL, Watanabe M, Haverty TP, Ziyadeh FN: Role of insulin and IGF1 receptors in proliferation of cultured renal proximal tubule cells. *Biochim Biophys Acta* 1133:329–335, 1992
17. Basson MD, Modlin IM, Madri JA: Human enterocyte (Caco-2) migration is modulated in vitro by extracellular matrix composition and epidermal growth factor. *J Clin Invest* 90:15–23, 1992
18. Duncan MD, Korman LY, Bass BL: Epidermal growth factor primes intestinal epithelial cells for proliferative effect of insulin-like growth factor I. *Dig Dis Sci* 39:2197–2201, 1994
19. Simmons JG, Hoyt EC, Westwick JK, Brenner DA, Pucilowska JB, Lund PK: Insulin-like growth factor-I and epidermal growth factor interact to regulate growth and gene expression in IEC-6 intestinal epithelial cells. *Mol Endocrinol* 9:1157–1165, 1995
20. Qureshi FG, Tchorzewski MT, Duncan MD, Harmon JW: EGF and IGF-I synergistically stimulate proliferation of human esophageal epithelial cells. *J Surg Res* 69:354–358, 1997
21. Counts RS, Nowak G, Wyatt RD, Schnellmann RG: Nephrotoxicant inhibition of renal proximal tubule cell regeneration. *Am J Physiol* 269:F274–F281, 1995
22. Sponsel HT, Breckon R, Hammond W, Anderson RJ: Mechanisms of recovery from mechanical injury of renal tubular epithelial cells. *Am J Physiol* 267:F257–F264, 1994
23. Racusen LC, Monteil C, Sgrignoli A, Lucskay M, Marouillat S, Rhim JG, Morin JP: Cell lines with extended in vitro growth potential from human renal proximal tubule: Characterization, response to inducers, and comparison with established cell lines. *J Lab Clin Med* 129:318–329, 1997
24. Lin JJ, Cybulsky AV, Goodyer PR, Fine RN, Kaskel FJ: Insulin-like growth factor-1 enhances epidermal growth factor receptor activation and renal tubular cell regeneration in postischemic acute renal failure. *J Lab Clin Med* 125:724–733, 1995
25. Yap J, Tsao T, Fawcett J, Fielder PJ, Keller GA, Rabkin R: Effect of insulin-like growth factor binding proteins 1-6 in rat kidney. *Kidney Int* 48:402–411, 1995
26. Price GJ, Berka JL, Edmondson SR, Werther GA, Bach LA: Localization of mRNAs for insulin-like growth factor binding proteins 1-6 in rat kidney. *Kidney Int* 48:402–411, 1995
27. Furlanetto RW, Harwell SE, Frick KK: Insulin-like growth factor-I induces cyclin-D1 expression in MG63 human osteosarcoma cells in vitro. *Mol Endocrinol* 8:510–517, 1994
28. Rosenthal SM, Cheng ZQ: Opposing early and late effects of insulin-like growth factor I on differentiation and the cell cycle regulatory retinoblastoma protein in skeletal myoblasts. *Proc Natl Acad Sci USA* 92:10307–10311, 1995
29. Ravitz MJ, Yan S, Dolce C, Kinniburgh AJ, Wenner CE: Differential regulation of p27 and cyclin D1 by TGF-beta and EGF in C3H 10T1/2 mouse fibroblasts. *J Cell Physiol* 168:510–520, 1996
30. Dufourny B, Alblas J, Van Teeffelen HA, van Schaik FM, van der Burg B, Steenbergh PH, Sussenbach JS: Mitogenic signaling of insulin-like growth factor I in MCF-7 human breast cancer cells requires phosphatidylinositol 3-kinase and is independent of mitogen-activated protein kinase. *J Biol Chem* 272:31163–31171, 1997
31. Fan Z, Lu Y, Wu X, Deblasio A, Koff A, Mendelsohn J: Prolonged induction of p21Cip1/WAF1/CDK2/PCNA complex by epidermal growth factor receptor activation mediates ligand-induced A431 cell growth inhibition. *J Cell Biol* 131:235–242, 1995
32. Jakus J, Yeudall WA: Growth inhibitory concentrations of EGF induce p21 (WAF1/Cip1) and alter cell cycle control in squamous carcinoma cells. *Oncogene* 12:2369–2376, 1996
33. Xie W, Su K, Wang D, Paterson AJ, Kudlow JE: MDA468 growth inhibition by EGF is associated with the induction of the cyclin-dependent kinase inhibitor p21WAF1. *Anticancer Res* 17:2627–2633, 1997